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Award Number: DAMD17-98-1-8656

TITLE: Biological Basis for Chemoprevention of Ovarian Cancer

PRINCIPAL INVESTIGATOR: Andrew Berchuck, M.D.

CONTRACTING ORGANIZATION: Duke University Medical Center
Durham, North Carolina 27710

REPORT DATE: October 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1999	Annual (1-Oct-98 - 30-Sep-99)

4. TITLE AND SUBTITLE
Biological Basis for Chemoprevention of Ovarian Cancer

5. FUNDING NUMBERS
DAMD17-98-1-8656

6. AUTHOR(S)
Andrew Berchuck, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Duke University Medical Center
Durham, North Carolina 27710

E-MAIL:
berch001@mc.duke.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Since reduction of ovulation is protective against ovarian cancer, prevention may represent a feasible approach to decreasing mortality. To achieve a better understanding of the etiology of ovarian cancer, which will translate into more effective chemoprevention strategies, we have initiated a molecular epidemiologic study in North Carolina. The study will consider genetic susceptibility, reproductive/hormonal and other exposures and acquired genetic alterations. The award from the DOD has been supplemented by a grant from the NCI allowing us to increase the number of cases and controls to 700; and the study will now be population-based with subjects recruited from 48 counties of central North Carolina. Subjects are interviewed in their homes, rather than by telephone, and 50 women with ovarian cancer have been accrued. Blood and cancer samples have been collected and DNA extracted and molecular analyses will commence when larger numbers of samples are available. Because progestins have a potent apoptotic effect on ovarian epithelial cells, the use of levonorgestrel in chemoprevention of ovarian cancer is being explored in chickens and women. A chemoprevention trial is ongoing in chickens and we will begin a trial to determine whether levonorgestrel induces apoptosis in the ovarian epithelium of women undergoing oophorectomy.

14. SUBJECT TERMS
Ovarian Cancer

15. NUMBER OF PAGES
13

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

A Burdine 10/29/99

PI - Signature Date

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Introduction

Ovarian cancer is the fourth leading cause of cancer deaths among women in the United States. There are three potential approaches to decreasing ovarian cancer mortality: screening and early detection, more effective treatment and prevention. All of these avenues should be explored, but we believe that prevention represents the most feasible approach. The rationale for prevention is derived from epidemiologic studies that have examined the relationship between reproductive history, hormone use and ovarian cancer. It has been convincingly demonstrated that reproductive events which reduce lifetime ovulatory cycles are protective. Although most women are unaware of this protective effect, those who use oral contraceptive pills for more than 5 years or have 3 children decrease their risk of ovarian cancer by greater than 50%. The biological mechanisms that underlie the association between ovulation and ovarian cancer are poorly understood, however. Our multidisciplinary ovarian cancer research group has been actively involved in studies that seek to elucidate the etiology of ovarian cancer and to translate this knowledge into effective preventive strategies. Joint consideration of genetic susceptibility, reproductive/hormonal and other exposures, acquired alterations in oncogenes and tumor suppressor genes and protective mechanisms such as apoptosis is required to accomplish this goal. We have initiated a molecular epidemiologic study of ovarian cancer in North Carolina to address the complex etiology of ovarian cancer. In addition, we are actively involved in development of chemopreventive strategies. We have performed a study in primates that suggests that the oral contraceptive has a potent apoptotic effect on the ovarian epithelium, mediated by the progestin component. In addition, in subsequent studies performed *in vitro*, we have induced apoptosis in epithelial cells treated with the progestin levonorgestrel. Progestin mediated apoptotic effects may be a major mechanism underlying the protection against ovarian cancer afforded by OCP use. This forms the basis for an investigation of the progestin class of drugs as chemopreventive agents for epithelial ovarian cancer. Currently studies to test the progestin levonorgestrel are underway in chickens and women.

Body

Projects 1 and 3: Molecular-epidemiology of ovarian cancer

With the support of the Department of Defense Ovarian Cancer Research Program we have initiated a molecular epidemiologic study of ovarian cancer to work towards the goal of a better understanding of the etiology of ovarian cancer. Drs. Andrew Berchuck (Gynecologic Oncologist) and Joellen Schildkraut (Epidemiologist) are working together to lead this study. Our initial plan was to accrue frozen tumor tissue and blood from 500 epithelial ovarian cancer cases treated at Duke University, the University of North Carolina at Chapel Hill, East Carolina University and the Gynecologic Oncology Group Ovarian Cancer Tumor Bank. In addition, 500 age and race-matched control subjects were to be accrued and both cases and controls were to be interviewed by telephone regarding known risk factors for ovarian cancer. After funding to support this project was received from the Department of Defense with Dr Berchuck as PI, additional funding was received to support this project from the NCI with Dr Schildkraut as PI. The additional funding has allowed us to increase the scope of the study. We will now be having nurse interviewers visiting the homes of all the cases and controls to administer

the study questionnaire. The number of cases and controls we hope to accrue has been increased from 500 to 700 and the study will be population-based. Research subjects will be accrued from a 43 county region of North Carolina using a rapid cases ascertainment mechanism established through the state tumor registry (see outlined area on map in appendix). Thus far, the study has been approved by the IRBs at 50 of the 72 hospitals in the region (see newsletter in appendix). Treating physicians are contacted by mail to request permission to approach potential research subjects. A letter is sent inviting a woman to participate only if permission to contact is granted. Two nurse interviewers have been hired and trained and the research questionnaire was field tested on 20 women with ovarian cancer. Final revisions to the questionnaire were made before the study began to accrue actual research subjects. To date 50 women with newly diagnosed ovarian cancer have been interviewed for the study. The interactions between the nurses and subjects has been uniformly positive. The women with ovarian cancer are highly motivated to talk about their history and have a high level of interest in supporting a study aimed at increasing our understanding of the causes of ovarian cancer. They greatly appreciate the opportunity to talk with a nurse who is truly interested in hearing all the details of their life experience. Blood and cancer samples have been collected, but molecular analyses will commence after a larger number of samples have been obtained.

Previously, using ovarian cancer cases and controls from the CASH study, we found a strong association between high lifetime ovulatory exposure and alteration of the p53 tumor suppressor gene. In project 1 of this proposal, directed by Dr. Berchuck (Gynecologic Oncologist), we are seeking to confirm the association between high lifetime ovulatory exposure and alterations in p53. More broadly, we will attempt to demonstrate that alterations in specific genes (eg, p53, HER-2/*neu*) serve as molecular signatures of distinct etiologic pathways and allow definition of more homogenous subsets of ovarian cancer. This could be critical as we strive to develop prevention strategies, as the optimal means of prevention may vary between different subsets of these cancers. In project 2, directed by Dr. Futreal (Molecular Geneticist), we will examine the role of genetic susceptibility in the development of ovarian cancer. Although most of the genes responsible for dominant hereditary ovarian cancer syndromes (eg. BRCA1) likely have been discovered, there is evidence to suggest that polymorphisms in other genes may also affect cancer susceptibility in a more weakly penetrant fashion. Dr. Futreal will investigate whether genetic polymorphisms affect ovarian cancer susceptibility. Since the effect of cancer susceptibility genes may be modified by other genes and exposures, he will determine whether gene-gene and gene-environment interactions affect ovarian cancer susceptibility. Because of the low incidence of ovarian cancer, the ability to identify "high risk" subsets of women is critical if we hope to translate our emerging understanding of the etiology of ovarian cancer into effective prevention strategies.

Project 3: chemoprevention

Project 3 is under the direction of Gustavo Rodriguez, M.D. (Gynecologic Oncologist). The prevention strategy outlined in our proposal is based on the observation that progestins have a potent apoptotic effect on ovarian epithelial cells. With regard to cancer prevention, the apoptosis pathway is one of the most important *in vivo*

mechanisms that functions to eliminate cells that have sustained DNA damage and which are thus prone to malignant transformation. In addition, a number of well known chemopreventive agents have been demonstrated to activate the apoptosis pathway in the target tissues that they protect from neoplastic transformation. We have performed a study in primates that suggests that the oral contraceptive has a potent apoptotic effect on the ovarian epithelium, mediated by the progestin component. In addition, in subsequent studies performed *in vitro*, we have induced apoptosis in transformed, immortalized, cultured human ovarian epithelial cells treated with the progestin levonorgestrel. This suggests that progestins may have a direct apoptotic effect on the ovarian epithelium. The finding that progestins activate this critical pathway in the ovarian epithelium, the site where ovarian cancers arise, makes it likely that progestin mediated apoptotic effects are a major mechanism underlying the protection against ovarian cancer afforded by routine OCP use. This forms the basis for an investigation of the progestin class of drugs as chemopreventive agents for epithelial ovarian cancer.

The studies outlined in our prevention grant are designed to add further support to notion that progestins are potent apoptotic agents on human ovarian epithelial cells, and to directly test the hypothesis in an animal model that progestins confer preventive effects against ovarian cancer. These aims in the grant are: (1) to evaluate the apoptotic effect of progestins on the human ovarian epithelium *in vivo*, (2) elucidate the molecular mechanisms by which progestins induce apoptosis in ovarian epithelial cells, and (3) to directly test the hypothesis that progestins confer preventive effects against ovarian cancer in a chemoprevention trial in the chicken, the only animal species with a high incidence of ovarian cancer.

Aim 1: We are in the midst of a clinical pilot designed to demonstrate induction of apoptosis in human ovarian epithelial cells by progestins *in vivo*. The purpose of the study is to demonstrate that progestins have an apoptotic effect in the human ovarian epithelium similar to what we have observed in primates. The trial is a prospective, randomized, blinded, placebo controlled study, in which women undergoing planned removal of ovaries for benign indications undergo treatment with progestin or placebo for one month prior to surgery. The ovaries are examined for evidence of apoptosis in the ovarian epithelium, and the degree of ovarian epithelial cell apoptosis is compared in treatment versus control groups. During the past year, we have laid the groundwork for this clinical pilot, which is about to begin. We have developed a collaboration with a pharmaceutical company, which will provide levonorgestrel tablets and placebo for the study. An IND has been filed with and approved by the FDA for the investigational use of levonorgestrel in this study. We hope to complete this trial over the next 24 months.

Aim 2: We have begun to explore the mechanism(s) by which progestins exert an apoptotic effect on the ovarian epithelium. The discovery that progestins induce apoptosis in the ovarian epithelium led us to search for potential mechanisms of action underlying these apoptotic effects. It is possible that progestins induce the expression of factors in the ovarian stroma, which then induce apoptosis via a paracrine effect in the adjacent ovarian epithelium. Conversely, it is possible that progestins exert a direct apoptotic effect on the ovarian epithelium, mediated by the progestin receptor. In order

to identify target sites of action for progestin in the ovary, we first examined the normal human ovary for expression of the progesterone receptor. Immunohistochemical staining for progesterone receptor was performed on normal frozen ovarian tissue samples obtained from 40 women who underwent oophorectomy as part of a gynecologic procedure performed for benign gynecologic indications. The progesterone receptor was consistently expressed by the ovarian epithelium in all cases, including the epithelium from ovaries from both pre- and post-menopausal women. In addition, progesterone receptor expression was detected in the ovarian epithelium lining inclusion cysts trapped within the ovarian stroma. Progesterone receptor expression was absent in all non-epithelial areas of the ovary. Not surprisingly, we have found similar expression of the progesterone receptor in the ovarian epithelium in primates (cynomolgus macaque). We have further examined expression of progestin receptor in both non-malignant as well as malignant ovarian epithelial cells *in vitro*. We have demonstrated expression of both the A and B isoforms of the progestin receptor *in vitro* in several ovarian cancer cell lines (OVCA 420,429,432,433; OVCAR 3, DOV-13), as well in cell cultures derived non-malignant ovarian epithelium. Although the physiologic role of the progesterone receptor within the ovarian epithelium remains to be elucidated, localization of progesterone receptor to the ovarian epithelium suggests a functional role for progestins in ovarian epithelial cells.

Having noted expression of the progestin receptor in the ovarian epithelium, we have tested the hypothesis that progestins induce apoptosis in ovarian epithelial cells via a direct effect, mediated by the progestin receptor. We found that treatment with a variety of progestins, including the gonane, estrane and pregnane derivatives, induces apoptosis in cell cultures derived from non-malignant human ovarian epithelium as well as in several ovarian cancer cell lines. These data are the first demonstration of a direct apoptotic effect of progestins on non-malignant ovarian epithelial cells. We are in the midst of experiments designed to elucidate the molecular regulation of progestin-induced apoptosis in ovarian epithelial cells by TGF-Beta, p53, bax, and bcl2.

Aim 3: We are currently performing a two-year study in the domestic fowl; designed to test the hypothesis that progestin confers chemoprevention against ovarian cancer. In addition, our study will test the hypothesis that the protective effect of progestin is due to a biologic effect independent of ovulation. Our prevention trial will be completed in the year 2000. This project will take advantage of a long-neglected animal model to show the feasibility of prevention of ovarian cancer by progestins in women. Demonstration that progestin treatment confers chemoprevention in our avian animal model, particularly in hens who are anovulatory, would provide strong support of our hypothesis that the protective effect of contraceptive agents is due to a biologic effect caused by progestins, and not due to ovulation inhibition. More importantly, demonstration of a chemopreventive effect by progestin would then open the door for other similar avian chemopreventive trials, designed to identify the optimal progestins, in the optimal doses and schedules, as a prelude to consideration of chemopreventive trials in women.

We are the first group in the world to explore the use of the chicken as an animal model for studying ovarian cancer chemoprevention. The domestic hen is the only

animal species with a high incidence of spontaneous ovarian cancer, reaching a cancer incidence as high as 40% at six years of life. Presumably, the high ovulatory rate (daily) in the domestic hen leads to significant damage in the ovarian epithelium, and subsequently to a high risk of ovarian cancer. We have been accruing chicken ovarian cancers during this study, and have begun examining these cancers under the microscope. The normal chicken ovary is comprised of an outer cortex containing ova surrounding a medulla. A single layer of germanative epithelial cells overlies the cortex. Hundreds of small follicular cysts project from the surface. The surface epithelium is highly convoluted, with extensive invaginations and folds. Initial findings in our observations of chicken cancers indicate that these cancers form a tubular glandular pattern, are cytokeratin positive, and stain negatively for intracellular ovalbumin. In contrast, oviductal tumors, which also are common in the chicken, are cytokeratin negative, contain PAS+ eosinophilic droplets in the apical cytoplasm, and are ovalbumin positive. We have just recently submitted a grant to the Department of Defense in which we propose to carefully study the molecular alterations in chicken ovarian cancers that we will accrue in the prevention trial. We hope to gather evidence that chicken cancers are similar to human ovarian cancers thereby validating the use of this animal as a model for ovarian cancer prevention research.

Key research accomplishments

This project is in its early developmental stage. The results of these studies will mature and be reported in the future.

Reportable outcomes

None to date.

Conclusions

The studies initiated by our program will enable us to define more homogeneous subsets of ovarian cancer based on epidemiologic and molecular characteristics, to identify women who are at increased risk for this disease and to develop chemopreventive strategies designed to decrease ovarian cancer incidence and mortality.

References

None

Appendices

August 1999 —Vol. 1, No. 1
Graphic Designer—Nancy Rustam

North Carolina Ovarian Cancer Study

OVARIAN CANCER STATISTICS

- ♀ 25,000 new cases of ovarian cancer per year in the United States
- ♀ 14,500 deaths expected in 1999
- ♀ 4th leading cause of death from cancer in women
- ♀ Only 30% survive

PURPOSE OF NORTH CAROLINA STUDY

To identify the environmental, reproductive, and genetic factors that contribute to the development of ovarian cancer.

STUDY SPONSORSHIP

National Cancer Institute
Department of Defense

STUDY PERIOD

January 1999-2003

Are You on Board?

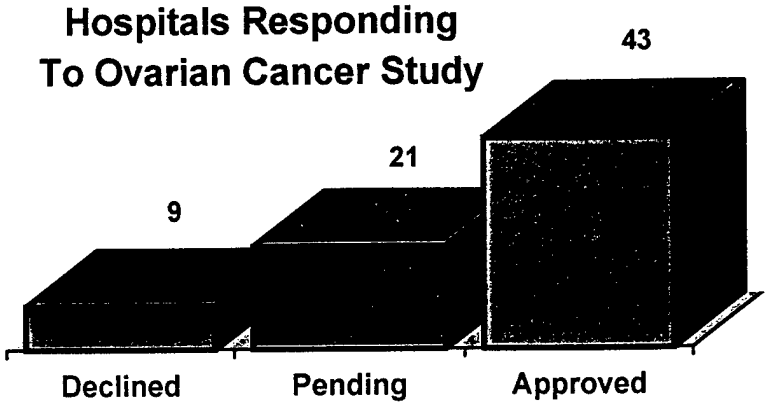
This comprehensive ovarian cancer study needs your help. In order to reach the ambitious population goal of 700 ovarian cancer patients and 700 cancer-free women, it is crucial to enlist the cooperation of as many physicians and hospitals as possible.

Ovarian cancer is a highly lethal disease that is the 4th leading cause of cancer deaths in women. This research study will collect epidemiologic data and perform molecular analyses on blood and tumor tissue. We believe that simultaneous consideration of epidemiology risk factors and molecular features will lead to a better appreciation of the factors that lead to the development of ovarian cancer.

In 48 counties throughout North Carolina, 73 hospitals and health care centers have been contacted, including yours. Of these, 43 have agreed to assist in identifying ovarian cancer cases and reporting these cases monthly to the North Carolina Central Cancer Registry.

Detailed study information has been sent to your hospital Cancer Registrar, and is under review. Let your Cancer Registrar and Institutional Review Board know how this valuable research can impact the lives of North Carolina women and women throughout the world. ♀

Number Of North Carolina Hospitals Responding To Ovarian Cancer Study



Number of Ovulatory Cycles As a Risk for Ovarian Cancer

Joellen Schildkraut, Ph.D., is an epidemiologist and one of the leaders of the North Carolina Ovarian Cancer Study. Her career has focused on studying risk factors for the disease such as family history and reproductive history. Andrew Berchuck, M.D., is a gynecologic oncologist who has been interested in defining the molecular alterations involved in the development of ovarian cancer. Over the past several years, they have teamed up and embarked together on molecular-epidemiologic studies of ovarian cancer.

In an initial study published in the Journal of the National Cancer Institute, they found that the more times a woman ovulates over her lifetime, the greater the chance that the tumor suppressor gene p53 will mutate and lead to ovarian cancer.

They tested for the presence of p53 mutations in ovarian tumor samples obtained from 197 ovarian cancer patients, aged 20-54.

Mutation of the p53 gene was indicated by accumulation of overexpressed mutant p53 proteins in ovarian cancer tissue. Women whose cancer was p53-positive had a greater number of lifetime ovulatory cycles than those whose cancers were p53-negative.

When data were controlled for age, menopausal status, and parity, women with p53-positive tumors were found to be significantly more likely to have had moderate or high numbers of ovulatory cycles than control subjects.

Dr. Schildkraut and her colleagues believe that further research is warranted to determine:

- Whether ovulation plays a different role in early-onset ovarian cancer compared to late-onset disease.
- How hormonal factors may influence ovarian cancer risk in the presence of molecular alterations.
- How multiple genetic influences may interact to increase risk of ovarian cancer development.

The findings from this study created the impetus for the North Carolina Ovarian Cancer Study currently being conducted. ♀

STUDY CONTACT INFORMATION

DUKE COMPREHENSIVE CANCER CENTER

- **Andrew Berchuck, M.D.**
  Professor, Division of Gynecologic Oncology
  (919) 684-3765
  email: berch001@mc.duke.ed

CANCER PREVENTION, DETECTION, AND CONTROL RESEARCH PROGRAM

- **Joellen Schildkraut, Ph.D.**
  Associate Professor
  (919) 681-4761
  email: schil001@mc.duke.edu
- **Mary Beth Bell, MPH**
  Project Manager
  1-888-246-1250
  (919) 681-4554
  email: mary.bell@duke.edu

NORTH CAROLINA CENTRAL CANCER REGISTRY

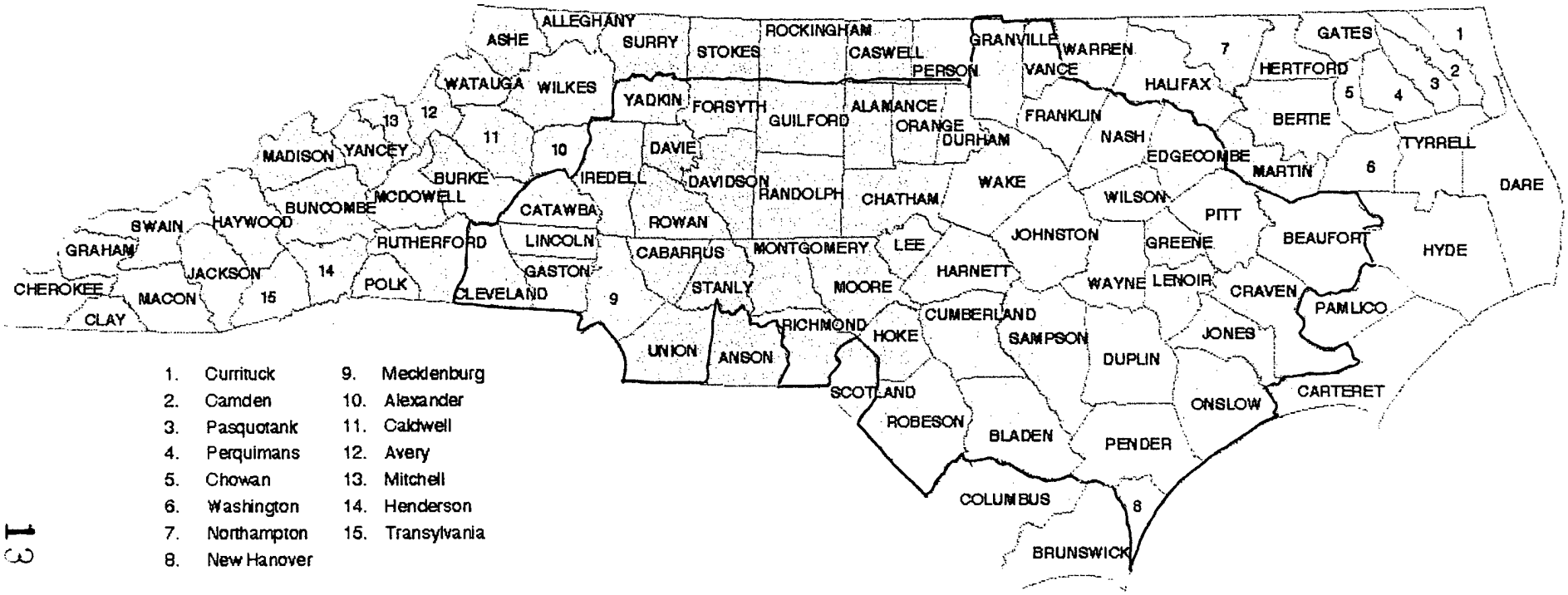
- **Rebecca Martin, Ph.D.**
  Director
  (919) 715-4555
- **Dianne Vann**, Research Associate
  (919) 715-4560

Study Participation—How It Works

- The hospital Cancer Registrar will send monthly information on newly diagnosed ovarian cancer cases to the North Carolina Central Cancer Registry. (If needed, a representative from the Cancer Registry can assist with this task).
- The Central Cancer Registry will forward potentially eligible cases to the study project manager for determination of study eligibility.
- A consent form will be sent to the attending physician requesting permission to contact their patient.
- When physician consent is received, a letter and brochure describing the study will be sent to the patient.
- Shortly thereafter, a nurse-interviewer will telephone the patient to discuss the study, determine eligibility, and invite those who are eligible to participate.
- Hospitals are paid $10 for every eligible case reported to the Central Cancer Registrar.

STATE OF NORTH CAROLINA

County Index Map



1. Currituck
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